ENDOTOXIN EXPOSURE IS A RISK FACTOR FOR ASTHMA: THE NATIONAL SURVEY OF ENDOTOXIN IN U.S. HOUSING

Peter S. Thorne, Ph.D.
Katarina Kulhánková, M.D., M.S.
Ming Yin, Ph.D.
Richard Cohn, Ph.D.
Samuel J. Arbes, Jr., D.D.S., M.P.H., Ph.D.
Darryl C. Zeldin, M.D.

ONLINE DATA SUPPLEMENT

METHODS

Study Design

This study was conducted using samples collected for the National Survey of Lead and Allergens in Housing. The study design, sampling, and endotoxin analysis methods have been published (E1). Briefly, the study was carried out in 831 housing units representative of the nation's 96 million homes that are permanently occupied, non-institutional, and that allow children as residents. There were four stages of survey design. First, 75 primary sampling units (PSUs) were selected systematically to represent the geographic regions, housing types, ethnic and socioeconomic characteristics and urbanicity of the United States. The second stage involved proportional population sampling of 754 segments within the 75 PSUs. The third stage was identification of 2 to 3 suitable housing units within each selected segment through field verification of eligibility. The final stage involved the selection of rooms and surfaces within homes for dust collection. If there was a resident child, the child's bedroom was sampled.

Assessment of Health Outcomes

Analyses included seven health outcome variables assessed at the individual level. These were ascertained as previously described (E1): diagnosed hay fever; diagnosed asthma; asthma symptoms past year; current asthma medication use; and wheezing ever, in the past month, and in the past year. Since exposure data were available by household, health outcomes were aggregated to the household level for primary analysis. Verification analyses were performed at the individual level.

Seven dichotomous disease outcomes were analyzed at the household level: diagnosed hay fever, diagnosed asthma, asthma symptoms past year, current asthma medication use, wheezing ever, in the past month, and in the past year. Household information on asthma and allergy was collected by an administered questionnaire. Diagnosed asthma was ascertained from the following question, "Has a doctor ever diagnosed anyone in your household with asthma including adults that had childhood asthma?" If answered in the affirmative, the respondent was asked questions about asthma symptoms, wheezing and medication use for each member of the household. Diagnosed asthma was defined as one or more persons in the household with asthma diagnosed by a doctor at anytime in their lifetimes (yes versus no, where no was no one in the household with diagnosed asthma). Symptomatic asthma was defined as at least one person in the household with doctor diagnosed asthma who had asthma symptoms in the past 12 months (yes versus no, where no was either no one with doctor diagnosed asthma or no diagnosed asthmatic with symptoms in the past 12 months). Other health outcomes including wheezing and medication use were assessed in a similar fashion. The respondent was also asked to provide allergy information for each household member. Diagnosed allergy was determined from the question, "Has a doctor ever diagnosed you (and other household members) with any allergies?" For each household member with doctor diagnosed allergy, the respondent was asked if the allergy was hay fever (allergic rhinitis), skin allergy, food allergy, or other allergies. Diagnosed allergy was defined as one or more persons in the household with at least one doctor diagnosed allergy (yes versus no, where no was no one in the household with diagnosed allergy). Hay fever was defined as one or more persons in the

household with diagnosed hay fever (yes versus no, where no was either no one with diagnosed allergy or no diagnosed allergic member with hay fever).

Sampling

Each household was visited by two field workers who administered a detailed questionnaire, conducted a home inspection, and collected samples. The questionnaire included information on demographics and health of the residents plus conditions of the home (E1). Dust was sampled by vacuum collection into an in-line filter using a standardized protocol. Dust was sieved (425 μm), aliquoted into 100 mg lots and then frozen at -80°C prior to indoor allergen and endotoxin assays.

Endotoxin analysis

Endotoxin analysis methods have been described previously (E1). Briefly, endotoxin was analyzed on 50mg quantities of sieved dust extracted with 1.0 ml pyrogen-free water containing 0.05% Tween-20. The kinetic chromogenic *Limulus* amebocyte lysate assay was employed with four two-fold dilutions of the samples and a 12 point standard curve referenced to endotoxin standard EC6 (E2). Selected samples were evaluated for inhibition or enhancement of the bioassay. The lower limit of detection for the assay was 0.001 EU/mg of sieved dust. All samples were assayed by a single analyst who was blinded to information on housing characteristics and asthma outcomes. Following linkage with housing unit and allergen data, 2512 endotoxin determinations were available for statistical analysis.

Additional statistical analysis

Allergen assays generally preceded the endotoxin assays and, in some cases, consumed all the collected dust. As a result, endotoxin determinations were performed

on a disproportionate number of samples from households that yielded higher quantities of dust. In order to assess the potential for bias, a set of 85 samples from kitchen floors were assayed from households that yielded very low quantities of dust (<100 mg). This evaluation demonstrated no significant relationship between the quantity of dust recovered in sampling and the concentration of endotoxin in the dust ($p=0.81$) obviating concerns about bias due to disproportionate use of higher quantity dust samples.

We performed supplemental analyses in order to the test the effect of aggregation at the household level with imputation of missing values. This was done by analyzing exposure-outcomes data at the individual level for all subjects by creating a house index and imputing missing bedroom floor endotoxin values from available data from other rooms in the house. To impute values, we fit a series of regression models where the bedroom floor endotoxin values were modeled based on endotoxin values from other sites available in the same household. Where we had values for at least three sites (e.g. bedroom floor, bedding, and family room floor) we fit a model to predict bedroom floor endotoxin based on the other two. We fit such models for all combinations of sites, predicting bedroom floor endotoxin as a function of whatever other sites were available. Taking into account all possible room combinations, there were 15 regression models defined for the bedroom floor. To test the utility of the imputation, we then fit each model and "predicted" the actual bedroom floor data that we had measured. For all models we found that between 75 and 80% of predicted (imputed) concentrations were correctly classified as above or below the stratification threshold. Then using the R-square values to rank the models we selected the model yielding the highest R-squared for which other data were available. That particular

regression model was used to impute values for all the missing bedroom floor data. Since these models were based on all available data, they exceeded the predictivity of the validation models based described above. This same method was then used to impute values for the bedding. For the bedroom floor 202 households out of 831 were imputed, and for the bedding 320 households were imputed.

RESULTS

Additional results are provided in this online supplement. Table E1 presents raw geometric mean, geometric standard error, 95% confidence intervals, and 5^{th} and 95^{th} percentiles for endotoxin concentration and loading for the five surfaces sampled. This complements the weighted values provided in the manuscript. The prevalence of lifetime asthma diagnosis observed in this study as compared to the National Health Interview Survey (E3) is shown in Table E2 and illustrates that this study represents national values for diagnosed asthma overall and as well as for adults and children, and men and women.

The relationship of endotoxin concentration to endotoxin load is plotted one against the other separately for the sampling locations in Figure E1. This shows a linear relationship between the logarithms of the two variables with correlation coefficients of 0.73 for the bedroom floor samples and 0.79 for the bedding samples. The coefficient for family room floor samples was also 0.73 (not shown). These data suggest that either concentration or load could be used as measures of household endotoxin. Since the mass of dust collected was known with greater accuracy than the area sampled,

particularly for sofa and kitchen floor, we performed our analyses using endotoxin concentration data.

Table E3 provides weighted Pearson correlation coefficients between pairs of household sites for log endotoxin load. The correlation coefficients for endotoxin concentration are provided in the manuscript. Odds ratios and 95% confidence intervals are presented in Table E4 from individual level logistic regression models separately for adults and children for the presence of health outcomes using bedroom floor endotoxin exposure levels above and below the first quartile. Thirty-five percent of the subjects in this analysis were less than 18 years old. Table E5 provides data for the relationship between bedroom floor and bedding endotoxin concentration and self-reported doctor-diagnosed asthma, asthma symptoms (past 12 months) and wheeze (past 12 months) with stratification by allergy status at the household level. Allergy status was based upon self-reported doctor-diagnosed allergy. This analysis had reduced power due to lower subject numbers and additional stratification. However, the adjusted odds ratio for wheeze in the past month was higher for households with allergic residents that had higher endotoxin as compared to those with non-allergic asthma and higher endotoxin. This analysis demonstrated no significant interaction between health outcomes and allergy status.

REFERENCES

E1. Vojta PJ, Friedman W, Marker DA, Clickner R, Rogers JW, Viet SM, Muilenberg ML, Thorne PS, Arbes SJ Jr, Zeldin DC. First national survey of lead and allergens in housing: survey design and methods for the allergen and endotoxin component. *Environ Health Perspect.* 2002;110:527-32.

E2. Thorne PS. Inhalation toxicology models of endotoxin and bioaerosol-induced inflammation. *Toxicology.* 2000;152:627-31.

E3. NCHS 2002. National Center for Health Statistics (U.S.). Asthma prevalence, health care use and mortality, 2002. (Assessed February 11, 2005, at http://www.cdc.gov/nchs/products/pubs/pubd/hestats/asthma/asthma.htm.)

FIGURE LEGEND

Figure E1. Correlation plots comparing endotoxin concentration to endotoxin load for bedroom floor and bedding dust. Correlation coefficients were 0.73 and 0.79, respectively, showing a high degree of correlation. Six values that appear to be outliers were verified through reanalysis of endotoxin in the samples.

Table E1. Geometric mean and distribution of endotoxin concentration and loading for the five surfaces sampled. Values are unweighted.

Sampled Surface	Number	GM (GSE) 95% CI [§]	5th percentile	95% percentile
Endotoxin Concentration, EU/mg				
Bedroom Floor	588	37.7 (1.05) 34.1-41.6	5.0	264
Bedding	470	20.6 (1.07) 18.2-23.3	2.0	149
Family Room Floor	489	71.1 (1.05) 64.3-78.7	11.9	443
Family Room Sofa	468	45.3 (1.06) 40.7-50.5	6.1	244
Kitchen Floor	454	84.4 (1.06) 75.6-94.2	9.9	568
Endotoxin Load, EU/m^2 or EU/sample*				
Bedroom Floor	585	11,166 (1.06) 9,877-12,623	1,282	136,224
Bedding	463	4,529 (1.07) 3,966-5,172	426	40,868
Family Room Floor	488	20,093 (1.07) 17,715-22,790	2,546	215,727
Family Room Sofa*	468	20,146 (1.07) 17,698-22,932	2,678	200,053
Kitchen Floor*	454	19,193 (1.08) 16,397-22,464	918	268,548

[§]Geometric mean, geometric standard error in parentheses, and 95% confidence intervals.

Table E2. Prevalence of self-reported, doctor-diagnosed asthma and hayfever observed in this study as compared to the 2002 National Health Interview Survey (E3).

	Asthma Prevalence %		Hayfever Prevalence %	
	This Study	NHIS	This Study	NHIS
Overall	11.3	11.1	16.3	9.2
Children	11.7	12.2	11.9	10.3
Adults	10.9	10.6	18.2	8.9
Males	9.8	10.6	14.1	8.8
Females	12.6	11.6	18.2	9.6

Table E3. Weighted Pearson correlation coefficients between pairs of household sites for log endotoxin load.

Endotoxin Load	Bedroom Floor	Bedding	Family Room Floor	Family Room Sofa	Kitchen Floor
Bedroom Floor	1.00				
Bedding	0.42	1.00			
Family Room Floor	0.41	0.31	1.00		
Family Room Sofa	0.35	0.30	0.32	1.00	
Kitchen Floor	0.36	0.24	0.31	0.22	1.00

Table E4. Adjusted odds ratios and 95% confidence intervals from individual level logistic regression models for the presence of health outcomes using bedroom floor endotoxin exposure levels above and below the first quartile.

Disease outcomes	Endotoxin category, EU/mg	Adults		Children	
		Odds ratio[a]	95% CI	Odds ratio[a]	95% CI
Diagnosed hay fever	≤16.6	1.00		1.00	
	>16.6	1.13	0.62, 2.06	0.57	0.20, 1.61
Diagnosed asthma	≤16.6	1.00		1.00	
	>16.6	1.32	0.64, 2.73	0.91	0.27, 3.05
Asthma symptoms past year	≤16.6	**1.00**		1.00	
	>16.6	**4.08**	**1.52, 10.94**	0.72	0.19, 2.76
Current asthma medication use	≤16.6	**1.00**		1.00	
	>16.6	**4.25**	**1.17, 15.43**	0.82	0.21, 3.13
Wheezing, ever	≤16.6	1.00		1.00	
	>16.6	1.97	0.98, 3.97	0.72	0.31, 1.64
Wheezing, past month	≤16.6	1.00		1.00	
	>16.6	1.82	0.71, 4.65	1.12	0.24, 5.11
Wheezing, past year	≤16.6	1.00		1.00	
	>16.6	1.76	0.77, 4.02	0.76	0.27, 2.19

[a]Models are adjusted for census region, season when sampled, frequency of indoor cigarette smoking, education, poverty, ethnicity, race, a resident child less than 6 years, log(Der p + Der f), log(Can f), and log(Fel d). For asthma outcomes n=1008 to 1051 for adults and n=525-557 for children.

Table E5. Relationship between bedroom floor and bedding endotoxin concentration and self-reported doctor-diagnosed asthma, asthma symptoms (past 12 months) and wheeze (past 12 months) with stratification by allergy status at the household level. Allergy status was based upon self-reported doctor-diagnosed allergy.

		Diagnosed Asthma			**Asthma Symptoms**			**Wheeze, Past Month**		
Allergy Status	Endotoxin EU/mg	Adjusted OR	95% CI	P value for inter-action	Adjusted OR	95% CI	P value for inter-action	Adjusted OR	95% CI	P value for inter-action
Bedroom Floor Endotoxin[a]										
Yes	≤ 16.6	1.00			1.00			1.00		
	> 16.6	1.26	0.49-3.23	0.97	2.38	0.78-7.28	0.94	2.21	0.80-6.11	0.37
No	≤ 16.6	1.00			1.00			1.00		
	> 16.6	1.29	0.40-4.16		2.57	0.51-13.0		1.10	0.39-3.11	
Bedding Endotoxin										
Yes	≤ 19.6	1.00			1.00			1.00		
	> 19.6	1.68	0.74-3.83	0.88	0.99	0.18-5.35	0.79	2.16	1.12-4.15	0.11
No	≤ 19.6	1.00			1.00			1.00		
	> 19.6	1.55	0.50-4.81		1.82	0.45-7.34		0.80	0.33-1.92	

[a]For bedroom floor endotoxin and health outcomes n = 480. For bedding endotoxin and health outcomes n = 383.

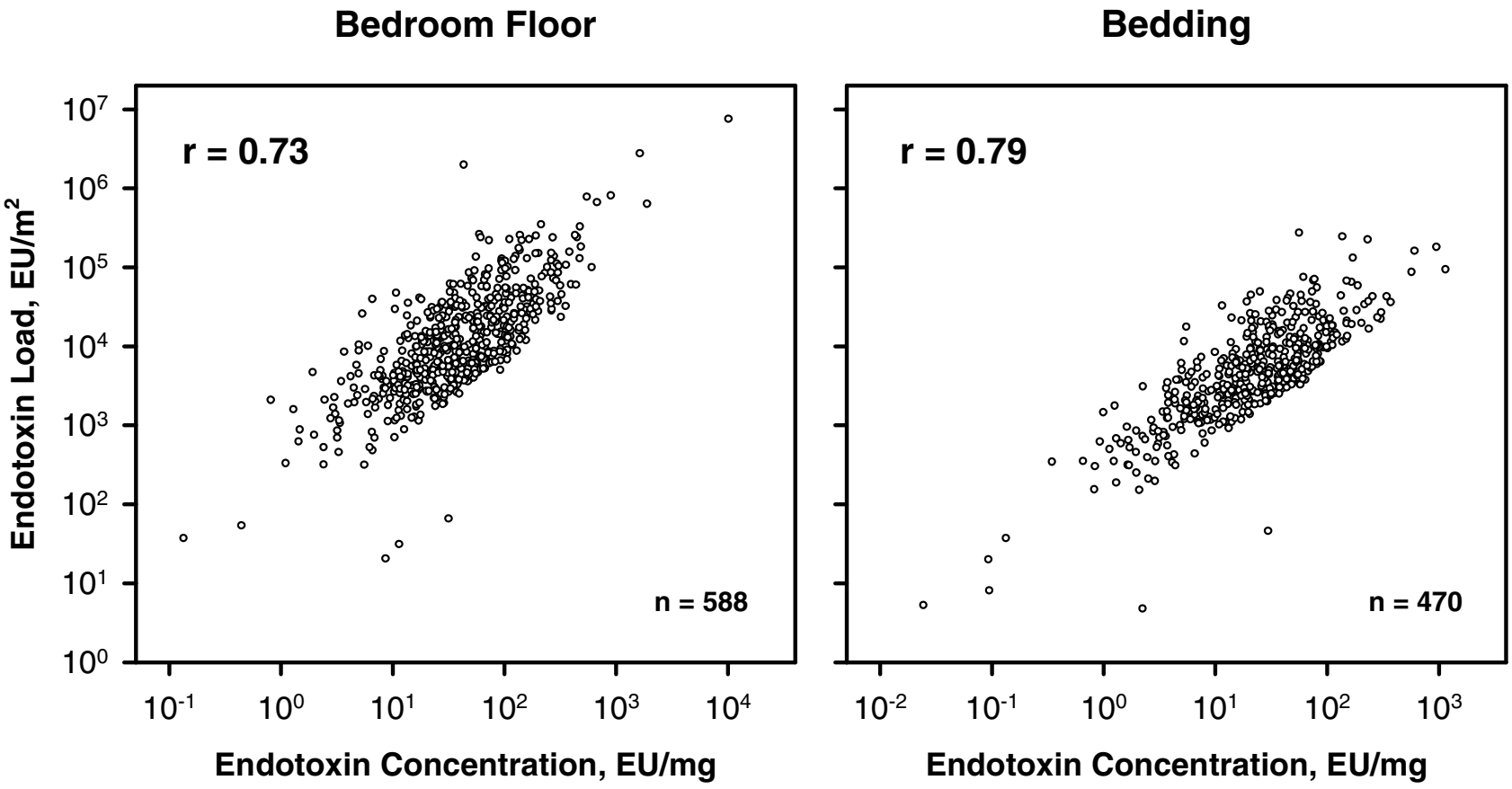
Bedroom Floor
Bedding
r = 0.73
r = 0.79
n = 588
n = 470
Endotoxin Load, EU/m²
Endotoxin Concentration, EU/mg
Endotoxin Concentration, EU/mg

Figure E1.